TEMPERATURE AND CRITICAL ILLUMINATION FOR
REACTION TO FLICKERING LIGHT

IV. ANAX NYMPHS

BY W. J. CROZIER AND ERNST WOLF
(From the Biological Laboratories, Harvard University, Cambridge)

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I

The first experiments testing the dependence of the form and other
properties of the flicker response contour upon temperature failed to
disclose any simple relation between critical intensity and temperature.
They did demonstrate that for the sunfish Enneacanthus and for the
nymphs of the dragon-fly Anax there occurs no appreciable change of
the maximum \( F_{\text{max}} \) over the range 12.4 to 27.3°C; and that the shape
of the \( F - \log I_m \) curve is not influenced by the temperature. The
general theory of the interpretation of the flicker response contour
requires that the first derivative of \( F \) vs. \( \log I_m \) with respect to \( \log I \)
give a frequency distribution of the elements of effect concerned in the
determination of the threshold response (this is not a frequency dis-
tribution of \( \log I \) thresholds for the receptor or central units providing
these effects); at a given \( F \), \( I_m \) is the mean intensity required for the
activation of a summed total number of the sensory elements sufficient
to cause the response to occur. If these elements form at any instant
a frequency distribution of thresholds of excitability which is “nor-
mal,” then the frequency distribution of \( d(1/I) \) should be normal; if
these elements fluctuate in their capacity to contribute to the deter-
mination of the result measured, then, a finite time being involved in
the process of excitation, the distribution of \( d(-\log I) \), or of \( d(\log I) \)
for the effects produced will be normal. The curve of \( F - \log I \) will

20, 393; 1936–37 b, 20, 411.
be a probability integral. In structurally uncomplicated cases this is the fact. In duplex visual systems the separation of the constituent populations of sensory effects is rationally made and tested analytically on this basis. The behavior of the parameters of the probability integral, with respect to number of retinal elements (area), and to light-time fraction in the flash cycle, is entirely consistent with this conception. So also is the qualitative fact that with elevation of temperature the $F - \log I$ contour is simply moved to a lower place on the temperature scale, without change of shape or change of maximum ordinate. Elevation of temperature simply increases the excitability $(1/I)$ of each elementary unit concerned; it cannot be held to modify the magnitude of the contribution made by an element to the determination of the response, since neither the shape nor maximum of the $F - \log I$ contour is modified. This also supplies a functional proof that the elements in a population behaving in this manner do in fact constitute a homogeneous population: the processes governing excitability are of the same kind in all the elements of such a population, since their arousal is influenced by temperature to quantitatively the same proportionate extent.

It was shown that for the turtle *Pseudemys* the value of $1/I$ for two fixed levels of $F$ follows the rule obeyed by the velocities or frequencies of very many biological processes, including among other things such phenomena as the reciprocals of the latent times for photic responses with $I$ constant. The reciprocals of the critical intensities

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Hecht, S., 1925–28, *J. Gen. Physiol.*, 8, 291; 1926–27, 10, 781, etc.
for reaction to flicker measure the speeds of the "driving" processes leading to the eventuation of the index response. This is the determination of levels of excitability governing the measured value of the flash illumination responsible for reaction to flicker at a given flash frequency. The Arrhenius equation describes the dependence of \(1/I\) upon temperature, and with values of \(\mu\), the temperature characteristic or apparent critical increment, which are found in a number of other biological processes.

When the \(F - \log I_a\) curves for sunfish and \(Anax\) were examined at three temperatures it was noted\(^1\) that instead of finding \(\log (1/I)\) a rectilinear function of reciprocal absolute temperature, as required by the Arrhenius equation, the plot was concave upward. This could easily result from the complexity of the process measured, if two or more concurrent processes should be simultaneously concerned in determining the sensitivities of the individual elements of excitability. It was accordingly suggested\(^1\) that the phenomena of response to flicker were probably too complex for treatment in a simple, direct way as a function of temperature. The results with \(Pseudemys\)\(^2\) showed that this conclusion was probably too superficial. It should indeed have been recognized\(^2\) that measurements at three temperatures could not give a clear result in the event that their span includes a critical temperature.\(^11\) On either side of a critical temperature there may obtain a different slope constant (\(\mu\)) for dependence upon temperature, or a change in velocity without change of \(\mu\). The former occurs in the data from \(Pseudemys\).\(^3\) The peculiar feature of the measurements with the sunfish, an apparent increase of \(\mu\) with rising temperature, has been resolved by a careful re-examination of this case.\(^12\) It turns out that one of the rare occurrences of a higher \(\mu\) on the higher temperature side of a critical temperature (ca. 20\(^\circ\)) was responsible for our original deductions,\(^1\) and that in fact \(1/I\) behaves as it should if governed by the velocity of reactions in a catalytic chain of which one or the other of two catalytically different steps is in control, depending on the temperature range.

\(^{11}\) Crozier, W. J., 1924-25, *J. Gen. Physiol.*, 7, 123, 189; 1925-26, 9, 525.  
II

In the meanwhile a good deal had been learned about the nature of the flicker response contour as obtained with arthropods. Forms such as *Anax*, *Cambarus*, *Uca*, *Apis*, *Drosophila*, provide curves of visual excitability (flicker, visual acuity) which depart in a consistent manner from the probability integral formulation. This has been traced to the fact that these arthropods have large, convex optic surfaces. The departure is consistent with the view that conditions of test which demand the action of a higher intensity of illumination permit the involvement of ommatidia further around the margin of the eye than can be stimulated under conditions of lower illumination. Up to a certain level of intensity, consequently, conditions (such as increase of flash frequency) which necessitate the use of higher intensities for the response, result in a virtual enlargement of the effective retinal area, and thus of the total number of neural elements implicated, and so lead to an augmentation of the level of critical effect (and of 1/I) at the point of response. Beyond a certain level of intensity this effect is no longer a factor and the \( F - \log I \) data beyond this point adhere to the typical probability curve. This conception has been tested, with concordant results, through the effects obtained by blocking out parts of the retinal surface in *Anax*, and in *Cambarus*, and by altering the proportion of light time to dark time in the flash cycle. It is also supported by the fact that in an arthropod with the most highly convex eyes (*Cambarus*) the distortion of the \( F - \log I \) curve is more extreme; and particularly by the fact that in a sufficiently flat-eyed form (*Asellus*) no distortion of this kind appears at all.

It is consequently of peculiar interest to re-examine the dependence of the *Anax* \( F - \log I \) curve upon temperature with the utmost care and precision possible. The data thus far available indicate unmistakably that the curve of \( \log 1/I \) is probably concave upward. We have considered that the shape of the curve could not be said to be significantly altered by temperature, although we have stated the fact that the crude temperature coefficient of \( 1/I \) is, as the data stood, to

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some extent a function of the flash frequency. The special significance of this case arises in two ways. If the effect referred to be real, we could perhaps obtain a quantitative key to the rôle of intrinsic excitability of the peripheral units in the determination of the form of the $F - \log I$ contour. In the second place, if it should appear that, apart from this effect, the Arrhenius plot of $1/I$ vs. $1/T_{ab}$ is unequivocally concave upward, rather than composed of two intersecting straight lines, as in the case of the sunfish, we would be in possession of one of the really rare instances among many biological phenomena thus far examined for which this special condition is to be observed. The theoretical interest of such cases is considerable; their analysis should present no particular difficulty, and is of first-rate importance for the theory of temperature characteristics. Experience over a period of some years has demonstrated that our sources of Anax larvae provide animals exhibiting a number of features of quantitative consistency with respect to the properties of measurements significant in such an inquiry. The effect sought is in one sense comparatively slight, as could be expected theoretically (see Section IV); the infrequent occurrence of instances of the kind found in the data on Enneacanthus led us to expect that this particular type of dependence on temperature might not, as a matter of sheer probability, occur in Anax as well. Certain purely mechanical considerations, already referred to, reinforced this expectation. Our experiments have accordingly been planned in a manner calculated to provide a critical test of the curvilinear or rectilinear character of the data to be obtained with Anax when displayed upon an Arrhenius grid.

III

At closely spaced intervals of temperature, with a homogeneous group of individuals, measurements were made of critical intensities for response of Anax nymphs to visual flicker at each of two flash frequencies, $F = 20$ and $F = 55$. The first flash frequency is at the center of the region of the $F - \log I_{m}$ curve which departs from the probability integral curve; the second is in the upper, orthodox part of the curve. The time order of the temperatures used was arranged

to reveal drifts of excitability with time and experimental history during the observations; none were noted. The uniform technic of observation and of calculation of the entries in the tables has been discussed in some detail in earlier papers,\(^7\) and need not be repeated.

Table I contains observations drawn from our data secured with different lots of \textit{Anax} larvae over a period of several years, showing the kind of reproducibility

\textbf{TABLE I}

Mean critical flash intensities, and the P.E. of the dispersions, from observations on marginal response to visual flicker in different lots of ten individuals of \textit{Anax junius} nymphs, over a period of 4 years, at 21.5\(^\circ\), with light time fraction in the flash cycle = 50 per cent. Data from earlier reports\(^4\) and (boldface type) from the present experiment.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|}
\hline
$F = 20 \text{sec.}$ & \\
\hline
\text{log } I_m & \text{log P.E.} \\
\hline
2.4881 & 3.2248 \\
2.4850 & 4.9845 \\
2.4876 & 4.7237 \\
2.4799 & 4.7236 \\
2.4778 & 4.8494 \\
\hline
$F = 30 \text{sec.}$ & \\
\hline
2.7403 & 4.9216 \\
2.7449 & 3.4360 \\
2.7486 & 3.1225 \\
2.7418 & 4.8608 \\
2.7497 & 4.8976 \\
\hline
$F = 55 \text{sec.}$ & \\
\hline
1.7356 & 2.4538 \\
1.7071 & 3.8599 \\
\hline
\end{tabular}
\end{table}

obtained. There is, of course, no reason at all for expecting identity of critical intensities, or of intrinsic variability, in lots of individuals from different sources. The sort of difference shown by the data in Table I is also found in experience with other kinds of animals.\(^1\)\(^6\)\(^\text{a}\)\(^\text{b}\) At $F = 20$ the new observations ($I_m$) are consistently just a little below those obtained in 1935-36 (at the same season

of the year), and also at $F = 55$. This is of no importance for the subsequent analysis.

Table II contains values of mean critical intensities at respectively $F = 20$ and $F = 55$, at various temperatures. The temperatures

<table>
<thead>
<tr>
<th>$t^\text{corr.}$</th>
<th>$I_m$</th>
<th>$\log P.E_{I_1}$</th>
<th>$F_{20}$</th>
<th>$I_m$</th>
<th>$\log P.E_{I_1}$</th>
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<td>$4.1464$</td>
<td>$1.8467$</td>
<td>$2.1123$</td>
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<tr>
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<td>$4.0651$</td>
<td>$1.8411$</td>
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<td>$1.8341$</td>
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<tr>
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<td>$1.0800$</td>
<td>$3.1021$</td>
<td>$1.1129$</td>
<td>$3.5813$</td>
<td></td>
</tr>
</tbody>
</table>

listed are mean temperatures in the aquaria during the period of observation. The animals were dark adapted for several hours at a particular temperature, in a thermostat. The temperature of the water in the individual aquaria changes by several tenths of a degree at most during the observations, depending on the distance from room
temperature (ca. 21.5°). These deviations, adjusted to the mean interval of observation by thermometer readings, are probably less in the substance of the Anax nymphs. Experience with these animals, however, unmistakably indicates that the rate of adjustment of body temperature under the conditions imposed is so rapid that the effective temperature cannot be in any case far from that tabulated. Deviations of mean temperature of 0.2° could not possibly affect the direction of the interpretation which the data demand. The same ten individuals were used throughout the experiment. The behavior of these individuals, as objectively indicated by the variations of the measurements, was very similar.

IV

We have first to examine the plottings of log \(1/I_m\) as a function of \(1/T_{obs}\), with reference to rectilinearity. These are given in Fig. 1. It is apparent that, as found previously, the temperature coefficient for \(1/I\) does increase with rising temperature. The increase is smooth and regular. Thermodynamically this is impossible unless the net result (end-point effect) is determined by the summation of the effects of two or more concurrent processes, each proceeding independently over the whole range of temperature. Close inspection of the data shows, making all reasonable allowance for the variation of temperature at each point and the (constant) relative variability of \(1/I\) for each \(F\), that the course of the measurements in Fig. 1 cannot be described other than by a curve. In the somewhat (but only superficially) similar sunfish data the use of a curve rather than two separate lines is forbidden by the obvious properties of the data. The situation in the sunfish and turtle requires the assumption that at a critical temperature, 20° in the former case, 30° in the latter, there occurs a change from one pacemaker process to another; with the sunfish the controlling process above 20° has a higher \(\mu\) than that below; the “break” in the curve at the critical temperature is clean and definite. The mechanism of such changes presents a problem requiring separate discussion.

Fig. 1. $1/I_m$ for response of *Anax* nymphs to visual flicker, at various flash frequencies ($t_U = t_D$), as a function of temperature. Solid dots, data of Table II. Open circles, data from an earlier report. Log $1/I_m$ is not a rectilinear function of $1/T^\circ_{abs}$; the curve is continuously concave upward.
It is a striking fact in the large body of data having to do with temperature and the speeds or frequencies of biological processes that extremely few instances occur in which the temperature characteristic $\mu$ increases smoothly with rise of temperature, giving a continuously curved Arrhenius plot concave upward. Such a graph indicates plainly that at least two different processes are contributing simultaneously and independently to the governance of the index end-point. That such curves are found to be extremely rare is a powerful argument in support of the general proposition that rectilinearity in the Arrhenius plot properly signifies essential simplicity in the pacemaking reaction, this is supplemented by the fact that the constancy of the relative variation of performance requires the operation of a single rather than of a compound system of control. When the Arrhenius plot for over-all velocities in a purely chemical system is concave upward the method for analysis of the data is, however, perfectly straightforward and can be used to resolve the complexity of the situation with, in favorable cases, recognition of the contributing factors.

In the present data we have additional features providing internal confirmation of the applicability of the analysis.

V

The experiment was designed to reveal the occurrence of any change in the form of the $F - \log I$ curve produced by altering the temperature. One flash frequency ($F = 20$) was chosen near the center of that portion of the $\varepsilon\alpha x$ curve which departs widely from the probability integral, where the departure (in terms of $F$) is greatest. The other flash frequency ($F = 55$) was chosen on the part of the curve


which adheres to the probability integral. If $F_{\text{max}}$ is really unaffected by change of temperature, and if the shape of the curve is unmodified, then the difference between $\log I_n$ for $F = 20$ and $F = 55$ should be constant at all temperatures. This, of course, means statistically constant, since each $I_n$ carries with it a measure of the dispersion of the distribution of $I_1$’s from which it is obtained. In the lower portion of the $F - \log I$ graph the departure from the fundamental probability integral is easily modified, in Anax, by altering the light time fraction $s$ or the retinal area. The theory of the departure from the fundamental curve is that in the lower part of the graph increasing critical intensities involve slightly larger effective retinal areas, because of the convexity of the optic surface. The same result could, however, conceivably be brought about by increasing the photic excitability of the individual receptor units. It would not be surprising to find that increase of temperature should do this, so that the discrepant part of the curve should be made to approach the probability integral more closely. On the other hand, increase of temperature lowers the critical illumination at any fixed $F$, with retinal area constant, so that in the distorted part of the graph the net result might easily turn out to be a counterbalancing of the increasing sensitivity of the retinulae by the mechanically reduced efficiency of the critical illumination. A careful consideration of the variation of $I_1$ at $F = 20$ and at $F = 55$ should make it possible to detect the action of two such factors. If $F_{\text{max}}$ is found to be independent of temperature as it is in other instances not involving the matter of gross optic morphology it cannot be maintained that the total number of available sensory elements is affected.

The differences between $\log I_n$ for $F = 20$ and $F = 55$ (Table II) are plotted in Fig. 2. It is apparent that with the possible exception of the measurements at 35.8° the differences are in no sense significant. With this exception, the fluctuation in $\Delta \log I_n$ is noticeably greater at 15–16°C. than elsewhere, a fact not without possible meaning in view of the subsequent analytical discussion. Slight day-to-day differences in excitability do not influence these comparisons, inasmuch as the measurements at $F = 20$ and $F = 55$ at each temperature were made on the same day. The observations in one set at $F = 55$ for 35.8° required the use of critical intensities at a comparatively un-
TEMPERATURE AND RESPONSE TO FLICKER. IV

Fig. 2. As a test of the constancy of the shape of the $F - \log I_m$ curve with temperature varied, the interval $\Delta \log I_m$ between $\log I_m$ for $F = 20$ and $F = 50$ is plotted as a function of temperature. The dashed lines are drawn at $\pm 2 \times$ mean P.E.A. Only at $35.8^\circ$ is there suggestion of change. See text.

TABLE III

Mean critical intensities for response of *Anax* at $35.8^\circ$, at several flash frequencies; and mean critical flash frequencies; and mean critical flash frequencies at higher intensities; $t_L = t_D$.

<table>
<thead>
<tr>
<th>$F$/sec</th>
<th>$\log I_m$</th>
<th>$\log P.E.I_{F1}$</th>
<th>$\log I$</th>
<th>$F_m$</th>
<th>$P.E.I_{F1}$</th>
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<tr>
<td>30</td>
<td>5.1569</td>
<td>4.4017</td>
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<td></td>
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<td>40</td>
<td>5.3818</td>
<td>4.7126</td>
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<td>45</td>
<td>5.5143</td>
<td>4.7575</td>
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<td>50</td>
<td>5.7517</td>
<td>3.0318</td>
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<tr>
<td>55</td>
<td>1.0680</td>
<td>3.1021</td>
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</tr>
<tr>
<td></td>
<td>1.0730</td>
<td>3.7817</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>1.1129</td>
<td>3.5813</td>
<td></td>
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<tr>
<td>60</td>
<td>1.9681</td>
<td>3.1523</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>0.00</td>
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</tr>
<tr>
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<td></td>
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<td>61.35</td>
<td>0.299</td>
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<tr>
<td></td>
<td></td>
<td>61.35</td>
<td>0.257</td>
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<tr>
<td></td>
<td></td>
<td>61.65</td>
<td>0.316</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

fortunate steep place on our calibration charts of intensities in the apparatus; that this did not fundamentally influence either $I_m$ or

It is, however, made clear by the other tests made at the same $F$ with other lamps and their more favorable calibration curves (Tables II and III). It is indicated by the subsequent discussion that in all probability the departure of $\Delta \log I_m$ at $35.8^\circ$ is due to the exceptional behavior of $\log I_m$ at $F = 55$, rather than at $F = 20$; $\log I_m$ at $F = 55$ is 0.03 to 0.09 log unit lower than it "ought" to be, and $I_1$ is too variable.

The theory of the asymmetrical $F - \log I$ function for arthropods with quite convex eyes holds that, up to a certain intensity, increase of $I$ is able to recruit activity from the periphery of the optic surface, because of light leakage through the substance of the eye. At a particular flash frequency, therefore, such as $F = 55$, if the required critical intensity is reduced—as by raising the temperature—it should be possible to bring the intensity to a level such that the involvement of tangentially affected receptor units would be a significant factor. At temperatures up to $33^\circ$ there is no evidence that this is actually the case (Fig. 2). Examination shows that a temperature of $35-36^\circ$; however, brings $\log I_m$ just to the upper edge of the lower portion of the $F - \log I_m$ graph which at $21.5^\circ$ departs from the probability integral, so that additional recruitment of marginal elements might clearly be detectable. Since, however, determinations at other flash frequencies demonstrate a change in the form of the curve at $35.8^\circ$, it is necessary to appeal also to recruitment based upon the increase of peripheral excitabilities with rise of temperature. These data are contained in Table III. By comparison with determinations at $21.5^\circ$ it is seen that the differences $\Delta \log I_m$ between determinations at $21.5^\circ$ and at $35.8^\circ$ increase slightly but rather regularly around $F = 55$:

<table>
<thead>
<tr>
<th>$F$</th>
<th>$\Delta \log I_m$ (log $I_m$ at $21.5^\circ$) - (log $\Delta I_m$ at $35.8^\circ$)</th>
</tr>
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<tbody>
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<td>20</td>
<td>0.525</td>
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<td>30</td>
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</tbody>
</table>

It would not be unreasonable to find that with decreasing flash time ($1/F$) this effect could be decreased. As Table III shows, at $\log I = 1.50$ (corresponding to log $I = 2.1^+ at 21.5^\circ$) $F_m$ is 61.33 to 61.64 (mean = 61.475). This agrees very well with the corresponding 61.42 at 1.84 obtained at 21.5. The theoretic value of $F_{max}$ was there taken as 61.48; for the data at $35.8^\circ$ the same value is best; for the older measurements, at $21.5^\circ$, $F_{max}$ for the $I_m$ data

\[\sigma I_1,\]
was 61.1, and in the present series 60.9. The agreement must be regarded as quantitative; on a probability grid (Fig. 3) the slopes are identical, showing that $\sigma \log I$ for the underlying frequency distribution of sensory effects is not materially affected by temperature. P.E., Table III is of the order of magnitude found in earlier measurements, taking into account the fact that the curve is shifted on the log $I$ axis, but the values at $F = 60-61$ are a little high.

The lower part of the 35.8° graph, the discrepant portion, is, however, a little closer to that for 21.5° than the upper, straight part. The junction of the two segments of the graph (Fig. 3) is definitely more rounded, and runs to a higher $F$, at 35.8° than at 21.5°. This plainly indicates that in this part of the graph the lowering of critical intensity at fixed $F$ induced by raising the temperature has also brought with it a slight reduction in the effective retinal area. There is no sign that increased peripheral excitabilities have sufficed to really counterbalance
this effect. We are justified, however, in supposing that such a factor is operative to some extent. The evidence is mainly indirect. At constant temperature the variation of critical intensity and the mean critical intensity are in direct proportion. This is the relationship expected if $1/I$ is a measure of the driving force required to eventuate the response. Similarly, if a velocity of (chemical) change is a measure of the driving potential, we find the variation of physiological effects proportional to such a velocity to be directly proportional to the mean velocity. Clearly, if the variation is intrinsic to the reacting or performing living system concerned, the magnitude of the proportionality constant should bear some relationship to the complexity of the controlling events. Examples in which the variation of performance is still proportional to the measure of average performance, but with a different proportionality constant, have been encountered in studies of the relation between temperature and frequency or speed of activity, so that this general contention is not without factual support. Analogous instances are given by the geotropic orienting performance of young rats. In the present data we are concerned to discover if, with temperature as independent variable, the relation between $I_m$ and the variation of $I_1$ exhibits any differences at $F = 20$ and $F = 55$. We have indicated reasons for supposing in advance that at $F = 20$ the variation in performance should be greater. This should be reflected in a wider variance of the flash intensity required to enforce the index-behavior employed as an end-point. The greater variance is expected because, as increasing temperature reduces the required critical flash intensity ($F$ being fixed) it also reduces the chance of photic involvement of ommatidia around the periphery of the optic surface. At the same time it must be presumed that elevation of temperature will increase the excitability of these peripheral elements. So that we consider two opposing influences to be at work in the determination of the critical effect requiring motor response to the moving flashes. It does not require the assumption that this critical effect will be of the same magnitude or any energy scale to deduce that the variance of the critical intensity will be enhanced as a consequence. The standard by which this enhancement is to be gauged is provided by the observations at $F = 55$. For temperature above 33° the critical illumination falls above that for which (at 21.5°) the recruitment of marginally excitable ommatidia has a significant influence on the form of the curve. Hence, by comparison with the state of affairs at any constant temperature, the variation data for $F = 20$ must necessarily be expected to fall above those at $F = 55$. Fig. 4 demonstrates that this is indeed the fact. Except for $I_m$ at $t = 35.8°$, which for reasons already discussed shows excessive variation, the measurements at $F = 20$ exhibit a higher variation at all temperatures than do those at $F = 55$. With Anax larvae it has been found that the relative sensitivities of individuals in a lot of ten tend to be maintained for several hours. With certain other forms, e.g. various teleosts this is not the case. The effect is obliterated in Anax when parts of the eyes are covered. The reasons for this

have been discussed. It should be expected that at higher temperatures a larger proportion of the variance of critical intensity might be found to be "within individuals" as compared with "between individuals," despite the lowering of the critical intensity. Rise of temperature should accentuate the individual fluctuations of excitability, and decrease the chance of persisting individual differences. Examination shows that at 35.8° the mean rank order numbers for sensitivity are not retained in successive sets of readings, whereas there is distinct correlation at 21.5°. On the other hand the proportion of the total variance due to the differences between individuals, in a set of readings at one \( F \), is just as great as at lower temperatures.

Fig. 4. The variation of \( I_t \) at various temperatures (Table II), for two flash frequencies; solid dots, \( F = 20 \); open circlets, \( F = 55 \). Triangles give data from Table III. At \( F = 55, t = 35.8° \), the fluctuation of \( I_t \) is exceptional; otherwise, below \( F = 55 \) the variation is consistently higher than at \( F = 55 \) or above; see text.
It is to be clearly understood that the differences we have been discussing in this section are in one sense small. Their internal consistency in a group of very carefully controlled observations nevertheless shows objectively that a definite realistic dignity must be granted to them. Their analytic interpretation is an obligation which cannot be successfully avoided. They have an inescapable significance for the evaluation of any interpretive theory of the mechanism responsible for the observations. In the great majority of biological investigations this obligation is ignored.

Question may very properly be raised as to the rôle of intensity vs. quantity of light in a flash with reference to the implication of marginally excitable ommatidia. The facts show that when the flash time is reduced, with cycle time (i.e., frequency) constant, the critical intensity declines. From the data obtained by change of the light time fraction, with $F$ constant, one can compute for each flash intensity the flash time corresponding to the temperature which requires the same intensity; at $F = 20$ this flash time of course decreases as temperature rises (except at 33–36°C, the decline is almost rectilinear). However, without correction for the changing value of $F_{\text{max}}$, log $I_m$ declines more rapidly with decrease of $I_L$ at $F = 55$ than at $F = 20$. Hence it must predominantly be a matter of intensity rather than of quantity of light (either in a flash, or as an average during the comparatively prolonged interval required for observation) which determines the involvement of marginally situated ommatidia. This is, of course, quite consistent with the fact that Talbot's law has nothing to say as to the basis for response to flicker (i.e., at constant flash intensity $F$ increases as the light time fraction is reduced). The important point is that these considerations support the view that penetration of the tangential margins of the eye, in these arthropods, which is a function of intensity primarily, is the basis for the recruitment of additions to effective receptor area; this cannot be determined by mere modifications of peripheral excitability. The latter have, however, a subsidiary influence, as demonstrated by the second order effects at $F = 20$. The failure of $F_{\text{max}}$ to be in any way affected by temperature, over the range 8–36°C,

shows that the total number of available sensory elements, if at all influenced by temperature, must show a negligible temperature coefficient. It is only within the zone of intensities for which the recruitment effect is perceptible that the effect is detectable at all. The special effect of comparatively very high temperature at $F = 55$ is traced to the fact that $I_m$ is then brought within the zone of intensities for which the role of peripheral excitabilities is significant. The effect of exposure to $36^\circ$ is quickly and freely reversible, and therefore is not due to "injury."

The analysis of the dependence of $I_m$ upon temperature may then proceed with some confidence on the basis that $I_m$ at $F = 55$ is (save above $33^\circ$) uninfluenced by the effect of temperature upon peripheral excitability. The data further demonstrate (Section VI) that at $F = 20$ the influence of increased temperature in decreasing the critical intensity must rather exactly balance the opposite effects due to the decreased marginal penetrating action of the lowered intensity. The constant relative variation of $I_1$ over the range of temperatures, with $F = 20$ (Fig. 4), is itself a kind of proof of this.

VI

The plots in Fig. 1 show that log $1/I_m$ is related to $1/T^\circ_{abs}$ by a curve continuously concave upward. Writers who have been disposed to disapprove of the attempt to measure temperature characteristics of biological processes by means of the Arrhenius equation [velocity $\propto \exp. (-\mu/RT)$] have quite without exception failed to realize the significance of such cases, and the unusual rarity of their occurrence. Their importance was referred to in an early survey of biological temperature functions. They indicate unequivocally that more than one process contributes simultaneously to the determination of the observed end-point; their rarity unmistakeably implies that for the great majority of temperature-controllable organic processes simplicity of the governing mechanism is self-evident; this is entirely

supported by the properties of variation of performance in these processes. In chemical systems this is a commonplace, and the type of procedure required for further elucidation has been known for a long time. It is not without significance that the only definite biological instances of this sort previously known have involved the performance of systems in which it is quite obvious that at least two processes are concerned. This was indicated long ago for speed of parthenogenetic activation of echinoderm eggs by acid; a more elaborate demonstration is provided by Korr’s data on respiration of sea urchin eggs. The straightforward analysis of these data shows without question that, among other things, two different respiratory processes are here normally proceeding simultaneously. It is not to be understood that cases fail to arise in which two (or more) processes individually influenced in different ways by temperature may not provide an Arrhenius graph convex upward; a model instance of this type was carefully examined long since. The point is that a value of smoothly and continuously increasing with rise of temperature signifies the concurrent influence of two (or more) processes with different values of over the whole of the investigated temperature range.

Ideally one requires the possibility of experimentally isolating and in some way directly identifying the proposed contributory processes. This is not difficult in some cases. Even in the case of “simple” physicochemical reactions this is, of course, not always feasible; “wall reactions” complicating gas reactions provide a clearly analyzable type. For a situation such as the present one analysis can justifiably be made in a formal manner, with two ends in view: to provide a rational account of the data, and to supply a guide for subsequent tests. The rationality of the analysis is attested by internal properties of the data. The specific hypotheses resulting can be rather easily put to proof.

The analysis involves one basic consideration which is probably

30 To be given in another place.
strange, or at least unfamiliar. The discussion of curves of visual functions which are (in fact or ideally) symmetrical with log I as abscissa has customarily regarded the first derivative of the curve with respect to log I as a frequency distribution of excitabilities. This is quite incorrect. The derivative is a frequency distribution of effects produced as a consequence of the basic excitabilities. The fact that a statistical basis must be granted for these effects leads directly to the conclusion that the curve of total effect vs. log I must be a normal probability integral, regardless of the form of the frequency distribution of the instantaneous excitabilities of the individual contributing units (so long as their number is large). The proper measure of excitability is 1/I, not 1/log I. Consequently it is with 1/I that we must deal in considering the application of the equation for "energy of activation." This is the basis for testing the form of 1/I on an Arrhenius grid, by plotting log 1/I against 1/T°abs. (Fig. 3).

A formal resolution of a curvilinear graph in these coordinates, of course, cannot be proved to give a unique interpretation, even within the limits of statistical propriety. The method is one of trial. The physical limitations of a biological system force one to work within certain thermal limits. The slopes toward the ends of the curve (Fig. 3) provide suggestions as to the orders of magnitude of the two main contributory μ's. This does not require that there be only two such; of course it does not necessarily follow that the curve of the data can be constructed from two such processes alone. The construction of the curve of the data requires the selection of suitable μ's and also the selection of suitable relative positions on the log K axis for their graphs. While this at first sight may seem to provide large latitude in the choice of μ's, in fact it does not; with well determined values of K (i.e., of 1/I) the requirements of the curve are rigorous.

This process has been applied to the curves in Fig. 3. The result of many trials is given in Fig. 5. The points at 35.8° for F = 55 have been discounted, for reasons already discussed rather fully (Section V). With this exception the curves for F = 20 and F = 55 are sensibly

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identical in form. Two processes with respectively $\mu = 19,200$ and $\mu = 3,500$, and with velocities equal at ca. $15.9^\circ$, give a faithful account of the data. It has not been possible to find two others which do.

![Graph showing log $1/I_m$ vs $10^4/T_{abs}$](image)

**Fig. 5.** The measurements at $F = 20$ and at $F = 55$ are brought together on the log $1/I_m$ axis (cf. Fig. 1) by vertical displacement (data of $F = 55 \times$ antilog 1.205). The form of the two curves is the same (the exceptional points at $F = 55, t = 35.8^\circ$ are discussed in the text). The curve shows continuous increase of slope as the temperature rises, and thus of the temperature characteristic $\mu$. This signifies the concurrent participation of at least two independent processes with different $\mu$'s, determining the excitability ($1/I$) for response to flicker. The curvature found is accurately reproduced by the summation of the two processes whose velocity curves are shown below, one with $\mu = 19,200$, the other with $\mu = 3,400$. Their velocities are equal at $15.9^\circ$. 
It is to be remembered that the "velocities" are respectively to be measured by the proportionate contributions of the two processes to the determination of the end-point result, and they may well contribute in different ways.

In one subsidiary feature the curves in Fig. 5 are additionally suggestive. It has been pointed out that near a temperature at which there cross the curves for two opposed or parallel independent processes contributing to the governance of a particular result one must expect excessive variation of performance. It is probably not an accident that at 15-16° (Fig. 5) the scatter of mean $1/I_m$ is greater than elsewhere. (This has of course nothing to do with the relation between $I_m$ and $\sigma I_m$.)

Finally, confirmation of part of the basis for this type of analysis is independently obtainable from the data. The point involved is that if $1/I$ is the proper measure of excitability for a particular magnitude

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of sensory effect (i.e., of $F$ in this case), then, granted no change in the form of the $F - \log I$ curve, the difference between $1/I_m$ for $F = 55$ and for $F = 20$ must follow the same curve as a function of temperature as does $1/I_m$ for any given level of $F$. The argument is that the sensory excitation necessary to produce the constant increase of effect represented by the difference between reaction at $F = 20$ and at $F = 55$ will require a driving potential which is less the higher the temperature, and is measured by $1/I$. Thus whereas $\Delta \log I_m$ is constant (Fig. 2), the intensity difference required to produce this constant difference in sensory effect declines exponentially with rise of temperature—and in the present data the curve must have the same form as in Fig. 5. Fig. 6 shows that this requirement is satisfied; this of course follows if the log $1/I$ curves for $F = 20$ and $F = 55$ have the same shape as a function of $T_0^{0.0}$, so that $I_{55}$ and $I_{20}$ are in constant ratio.

VII

A limiting process with $\mu$ as low as 3,400 (Figs. 5 and 6) could be essentially one of hydrodiffusion, or it could represent the outcome of opposing processes in equilibrium. No certain deductions can be made with respect to it. In the present case it cannot be supposed that we have to do with a balance between the decreasing retinal area of operation of the critical flash intensity, on the one hand, and in the opposite direction the elevation of peripheral excitabilities brought about by rise of temperature, because (below 33°) above $F = 55$ the $F - \log I$ curve does not rise.

With respect to $\mu = 19,200$, however (Figs. 5 and 6) more definite notions may be entertained. A variety of evidence clearly suggests that it is associated with dehydrogenations (i.e., fundamentally, with activation of H+), and probably in a number of instances with the first steps in the burning of simple sugars. This may not be difficult to test, by way of suitably designed metabolic experiments. Thus by modification of the glycogen or other carbohydrate reserves, or of catalysts for dehydrogenation, the limiting role of the process for which $\mu = 19,200$ might be obliterated. In this manner clues might

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be available as to the nature of the events governing the capacity to react to flickering light. The temperature characteristics found\(^\text{17}\) for frequency of breathing movements in *Anax* nymphs (11,500 and 16,200) are quite different. It does not seem profitable to regard the chemical control of this visual excitability as located in the peripheral receptors, since in that event the enhancement of the "dark" process by elevation of temperature would be expected to have the effect of enlarging the population of elements of effect, just as reduction of the light time fraction does; this is not found.

**SUMMARY**

At fixed flash frequency \((F = 20, F = 55)\) and with constant light time fraction (50 per cent) in the flash cycle, the critical illumination \(I\) for response of *Anax* nymphs to visual flicker falls continuously as the temperature rises. The temperature characteristic \(\mu\) for the measure of excitability \((1/I)\) increases continuously with elevation of temperature. The form of the \(F-\log I\) curve does not change except at quite high temperature \((35.8^\circ)\), and then only slightly (near \(F = 55\)); \(F_{\text{max}}\) is not altered. The very unusual form of the \(1/I\) curve as a function of temperature is quantitatively accounted for if two processes, with respectively \(\mu = 19,200\) and \(\mu = 3,400\), contribute independently and simultaneously to the control of the speed of the reaction governing the excitability; the velocities of these two processes are equal at 15.9\(^\circ\).

We wish to express our thanks to Mrs. E. Wolf for her assistance in the experiments.